BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

Consommation et Affaires commerciales Canada
Bureau des brevets
Ottawa, Canada
K1A 0C9

Consumer and Corporate Affairs Canada
Patent Office

(11) (C) **1,322,162**

(21) 558,498

(22) 1988/02/09

(45) 1993/09/14

(52) 167-103.36
C.L. CR. 31-1.15
167-300

(51) INTL.CL.[5] A23L-003/34; A01N-063/02; A23K-003/00; A24B-015/30

(19)(CA) **CANADIAN PATENT** (12)

(54) Formulation for Extending the Shelf Life of Food Products, Medicaments and Cosmetic Products

(72) Lück, Erich , Germany (Federal Republic of)
Klug, Christian , Germany (Federal Republic of)
Lotz, Andreas , Germany (Federal Republic of)
Wöhner, Gerhard , Germany (Federal Republic of)
von Rymon Lipinski, Gert-Wolfhard , Germany (Federal Republic of)

(73) Hoechst Aktiengesellschaft , Germany (Federal Republic of)

(30) (DE) Germany (Federal Republic of) P 37 04 004.9
1987/02/10

(57) 11 Claims

NO DRAWING

Canada

CCA 3254 (10-92) 41 7530-21-936-3254

5558498

Abstract of the disclosure HOE 87/F 039

Combining non-enzymatic preservatives with enzymes having N-acetylmuramidase activity results in a synergism of action which is distinguished by the fact that, in particular, food products, medicaments and cosmetic products, as well as packaging materials, tobacco and tobacco products can be preserved with the mixture for comparatively longer than by using the individual components.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A formulation containing one or more non-enzymatic preservatives plus one or more enzymes having N-acetylmuramidase activity in a ratio by weight of 1 to 100 to 100 to 1.

2. A formulation as claimed in claim 1, wherein the non-enzymatic preservative and the enzyme having N-acetylmuramidase activity are mixed in a ratio by weight of 1 to 50 to 50 to 1.

3. A formulation as claimed in claim 1, containing either or both of benzoic acid and sorbic acid or their salts, plus either or both of lysozyme and bacteriolytic enzyme products from Streptomycetes.

4. A method of preserving easily spoiled goods which comprises administering to said food an amount of the formulation as defined in claim 1, which is effective to preserve said goods.

5. The method as claimed in claim 4, wherein the goods are food products, cosmetic or pharmaceutical products, packaging materials, tobacco and tobacco products, or animal feeds.

6. The method as claimed in claim 4, wherein the amount of the formulation added to the product to be preserved is such that it contains 0.01 to 2000 U of the enzyme having N-acetylmuramidase activity per mg.

7. The method as claimed in claim 6, wherein the content is 0.1 to 1000 U/mg.

8. The use of the formulation as claimed in claim 1, as a preservative in easily spoiled goods.

9. The use of the formulation as claimed in claim 8, wherein the goods are food products, cosmetic or pharmaceutical products, packaging materials, tobacco and tobacco products, or animal feeds.

10. The use of the formulation as claimed in claim 8, wherein the amount of the formulation added to the goods to be preserved is such that it contains 0.01 to 2000 U of the enzyme having N-acetylmuramidase activity per mg.

11. The use of the formulation as claimed in claim 10, wherein the content is 0.1 to 1000 U/mg.

HOECHST AKTIENGESELLSCHAFT Dr. KH/gm HOE 87/F 039

Specification

A formulation for extending the shelf life of food products, medicaments and cosmetic products

It is known to use preservatives such as, for example, sorbic acid, benzoic acid, propionic acid, formic acid, p-hydroxybenzoic acid, salicylic acid and sulfurous acid to extend the shelf life of food products, medicaments and cosmetic products. In the preservation of food products in practice the effect of the said preservatives is often intensified by additives which lower the water activity or the pH of the food products, such as sodium chloride, sugar, acetic acid or other edible acids. This may result in changes in the taste of the food products, which are not always desired. In the case of mayonnaises and marinated fish and other delicatessen products, the consumer prefers the products to be adjusted to a mild flavor, with low additions of salt and vinegar. However, there is the risk in the higher pH range that the growth of some bacteria will be intensified and even that favorable living conditions for pathogenic organisms are set up. Cases of food poisoning connected with the consumption of only weakly acidified products repeatedly occur, because the antimicrobial action of many preservatives decreases markedly with increasing pH.

Enzymes having N-acetylmuramidase activity such as, for example, lysozyme can likewise be used for the preservation of food products, for example milk, meat and fish products. The growth of microorganisms in milk products is prevented by preservation with lysozyme using the process in British Patent 2,014,032. The use of lysozyme-like products from secretions of animals and plants for preventing late bubble formation in various types of cheese is described in French Patent 8,003,321.

However, products of these types can be obtained not only from plants and animals but also from Streptomycetes, as is evident from German Offenlegungsschriften 2,011,935, 2,040,444, 2,146,597 and 3,440,735.

It has now been found, surprisingly, that combining the non-enzymatic preservatives with enzymes which have N-acetylmuramidase activity results in a synergism of action which is distinguished by the fact that, in particular, food products, medicaments and cosmetic products can be preserved with the mixture for comparatively longer than by using the individual components.

Hence the invention relates to:

1. A formulation containing one or more non-enzymatic preservatives and one or more enzymes having N-acetylmuramidase activity in a ratio by weight of 1 to 100 to 100 to 1.

2. A method of preserving goods using the formulation defined under 1.

3. A process for the preparation of the formulation defined under 1., which comprises converting the non-enzymatic preservative and the enzyme having N-acetylmuramidase activity into a suitable application form.

The invention is described in detail, especially in its preferred embodiments, hereinafter. The invention is also defined in the patent claims.

Examples of suitable preservatives used according to the invention as active substances in the formulation are sorbic acid, benzoic acid, propionic acid, formic acid, p-hydroxybenzoic acid, salicylic acid and sulfurous acid, as well as their salts. Particularly good results can be obtained with benzoic acid or sorbic acid or their salts. The second active component in the formulation is an

enzyme having N-acetylmuramidase activity and having the ability to lyse bacterial cells and to prevent further growth of the microorganisms. Examples of enzymes which are suitable and preferred are lysozyme, in particular from hen's egg white, or bacteriolytic enzyme products from Streptomycetes, in particular from Streptomyces coelicolor DSM 3030 and its mutants and variants. The enzyme product from DSM 3030 is readily obtained by the process described in German Offenlegungsschrift 3,440,735. In this process, Streptomyces coelicolor DSM 3030 is cultivated in a fermentation medium with the addition of sugarbeet molasses in an amount of 5 to 50 g, preferably 10 to 20 g, per liter of culture medium. A further increase in the yield is achieved by addition to the culture medium of calcium ions in the form of readily soluble, non-toxic calcium salts, preferably in the form of calcium chloride, which is reasonably priced. A calcium ion concentration of 0.05 to 1 molar is advantageous, and concentrations of 100 to 500 mmol/l are particularly preferred, for example in the form of addition of 0.2 to 0.5% by weight of calcium chloride dihydrate.

The active substances can be added simultaneously or successively to food products such as, for example, meat and meat products, fish, crustacean, shellfish and mollusc products, edible gelatinous coating compositions for meat products, delicatessan products, liquid egg and liquid egg yolk, vegetable and fruit products, alcoholic and non-alcoholic beverages, milk and milk products, fillings for pastries and bakery products, confectionery and candies, as well as pharmaceutical and cosmetic products, animal feeds, tobacco, tobacco products and packaging materials.

Depending on the intended use, one or more enzymes having N-acetylmuramidase activity are mixed with at least one of the non-enzymatic preservatives in a ratio by weight of 1 to 100 to 100 to 1, preferably 1:50 to 50:1, and added, by conventional processes, to the product to be preserved. The amount of the mixture added is of an order such that

0.01 to 2000 U of the enzyme having N-acetylmuramidase activity, preferably 0.1 to 1000 U, are contained in each mg of the product to be preserved.

The combination of chemical preservatives and enzymes having muramidase activity results in a synergism of action. This has a particularly advantageous effect on products with a mild pH, which are particularly favored by consumers. For example, the action of the combination in a pH range between 3 and 6, preferably 4.5 to 5.5, against microorganisms which spoil food products and are pathogenic is better than that of the individual components alone. The advantage of the mixture is that the content of chemical preservatives in food products, cosmetics, pharmaceuticals etc can be reduced while simultaneously extending the shelf life. It is self-evident that the combination of active substances according to the invention can or should be used only in the logical case where the products to be preserved have been manufactured under satisfactory hygienic conditions and have a low initial organism count.

The invention is explained further in the examples which follow. Unless otherwise indicated, percentage data relate to weight.

Example 1

Investigation of the shelf life of salad mayonnaise

A salad mayonnaise of the following recipe was prepared:

oil	50.0 %
water	31.1 %
starch	3.1 %
egg yolk	4.6 %
salt	1.0 %
sugar	4.0 %
guar gum	0.2 %

The pH of the salad mayonnaise was adjusted to pH 4.5 with acetic acid. The mixture was divided into four batches, one of which was left unaltered (I). The additions to the other portions were 100 U of lysozyme per mg (II), 50 U of lysozyme per mg + 0.15 % of potassium sorbate (III) and 0.2 % of potassium sorbate (IV) in each case.

The mixtures were stored in closed jars at a temperature of +10°C.

The shelf life was assessed on the basis of alterations perceptible with the senses and of the total organism count.

	Added preservative	Shelf life [days]
I	Control test	6
II	100 U of lysozyme	12
III	50 U of lysozyme/mg + 0.15 % of potassium sorbate	19
IV	0.2 % of potassium sorbate	14

Determination of the activity of bacteria-lysing enzyme product:

0.2 ml of samples containing bacteria-lysing enzyme product are pipetted to 2.8 ml of a suspension of 0.2 mg of Micrococcus luteus ATCC 4698 (Boehringer Mannheim) per ml of 0.1 M sodium acetate buffer (pH 5.0), and the decrease in turbidity is determined at 25°C by measuring the extinction at 450 nm. 1 unit is defined as the decrease in extinction by 0.001 photometer scale units per minute.

Example 2

Investigation of the shelf life of mayonnaise

A mayonnaise of the following recipe was prepared:

oil	82.0 %
water	10.0 %
sugar	4.0 %
mustard	2.0 %
salt	2.0 %

The pH of the mayonnaise was adjusted to 4.5 with acetic acid. The mayonnaise was treated, stored and assessed in analogy to Example 1. The shelf lives emerged as follows:

	Added preservative	Shelf life [days]
I	Control test	9
II	100 U of lysozyme/mg	15
III	50 U of lysozyme/mg + 0.15 % of potassium sorbate	23
IV	0.2 % of potassium sorbate	17

Example 3

Investigation of the shelf life of a meat/mayonnaise product

The salad mayonnaises I-IV prepared in Example 1 were each mixed with 40 % by weight of thinly sliced meat sausage and 20 % by weight of thinly sliced gherkins from one production batch. The meat/mayonnaise products were treated, stored and assessed in analogy to Example 1. The following shelf lives emerged:

	Added preservative	Shelf life [days]
I	Control test	3
II	40 U of lysozyme/mg	6
III	20 U of lysozyme/mg + 0.06 % of potassium sorbate	11
IV	0.08 % of potassium sorbate	8

Example 4

Investigation of the shelf life of salad dressing

A salad dressing of the following recipe was prepared:

water	34.1 %
oil	46.1 %
sugar	14.5 %
mustard	3.0 %
salt	2.1 %
xanthan	0.2 %

The pH was adjusted to 4.5 with acetic acid. The salad dressing was treated, stored and assessed in analogy to Example 1. The shelf lives emerged as follows:

	Added preservative	Shelf life [days]
I	Control test	6
II	100 U of lysozyme/mg	10
III	50 U of lysozyme/mg + 0.15 % of potassium sorbate	17
IV	0.2 % of potassium sorbate	12

Example 5

Investigation of the shelf life of a cream filling

A cake cream of the following recipe was prepared:

sugar	51.2 %
glucose syrup	25.6 %
water	22.0 %
functionalized protein	0.7 %
citric acid	0.4 %
agar agar	0.07 %
flavorings	0.03 %

The cream was divided into four batches, one of which was left unaltered (I). The additions to the other portions were 100 U of lysozyme per mg (II), 50 U of lysozyme per mg + 0.15 % of potassium sorbate (III) and 0.2 % of potassium sorbate (IV) in each case.

The mixtures were stored in closed jars at a temperature of +10°C.

The shelf life was assessed on the basis of alterations perceptible with the senses and of the total organism count.

	Added preservative	Shelf life [days]
I	Control test	5
II	100 U of lysozyme/mg	9
III	50 U of lysozyme/mg + 0.15 % of potassium sorbate	15
IV	0.2 % of potassium sorbate	11

Example 6

Investigation of the shelf life of a foamed fruit dessert product

A fruit dessert product of the following recipe was prepared:

water	74.6 %
icing sugar	18.8 %
modified starch	3.7 %
instant gelatine	1.2 %
fruit powder	0.7 %
citric acid	0.4 %
delta-gluconolactone	0.3 %
sodium bicarbonate	0.1 %
functionalized protein	0.1 %
flavorings	0.1 %

The fruit dessert product was divided into four batches, one of which was left unaltered (I). The additions to the other portions were 100 U of lysozyme per mg (II), 50 U of lysozyme per mg + 0.15 % of potassium sorbate (III) and 0.2 % of potassium sorbate (IV) in each case.

The mixtures were stored in closed jars at a temperature of +10°C.

The shelf life was assessed on the basis of alterations perceptible with the senses and of the total organism count.

	Added preservative	Shelf life [days]
I	Control test	6
II	100 U of lysozyme/mg	10
III	50 U of lysozyme/mg + 0.15 % of potassium sorbate	16
IV	0.2 % of potassium sorbate	11

Example 7

Investigation of the shelf life of a moisturizing cream

A moisturizing cream with the following formulation was prepared:

liquid paraffin (German Pharmacopeia)	10.0 %
vitamin oil	5.0 %
petrolatum (German Paharmacopeia)	5.0 %
®Hostacerin T-3 (fatty alcohol polyglycol ethers, emulsifier)	5.0 %
®Hostaphat KW 340 N (organic phosphoric acid derivative, emulsifier)	4.0 %
palmitic acid	5.0 %
cetyl alcohol (German Pharmacopeia)	1.0 %
water	60.0 %
®Hydroviton (humectant)	5.0 %

The cream was divided into four batches, one of which was left unaltered (I). During the preparation of creams II-IV, the following additions were metered into the aqueous phase:

cream II	250 U of lysozyme/mg
cream III	150 U of lysozyme/mg + 0.2 % of sorbic acid
cream IV	0.3 % of sorbic acid

The creams were stored in closed containers at room temperature.

The shelf life was assessed on the basis of alterations perceptible with the senses and of the total organism count.

	Added preservative	Shelf life [days]
I	Control test	9
II	250 U of lysozyme/mg	14
III	150 U of lysozyme/mg + 0.2 % of sorbic acid	30
IV	0.3 % of sorbic acid	19

Example 8

Investigation of the shelf life of a gel

A pharmaceutical gel with the following formulation was prepared:

water	97 %
hydroxyethylcellulose (Tylose H 1000)	3 %

The prepared gel was divided into four batches, of which one gel (I) remained untreated. The following additives were metered into gels II-IV:

gel II 250 U of lysozyme per mg
gel III 150 U of lysozyme per mg + 0.2 % of sorbic acid
gel IV 0.3 % of sorbic acid.

The gels were stored in closed containers at room temperature.

The shelf life was assessed on the basis of alterations perceptible with the senses and of the total organism count.

	Added preservative	Shelf life [days]
I	Control test	7
II	250 U of lysozyme/mg	19
III	150 U of lysozyme/mg + 0.2 % of sorbic acid	35
IV	0.3 % of sorbic acid	22

Example 9

Investigation of the shelf life of liquid rennet

Commercially available liquid rennet preserved with 1.1 % benzoic acid was used for the investigation of the shelf life. The liquid rennet was stored in closed containers at room temperature without and with the addition of 50,000 U of lysozyme per ml.

The shelf life was assessed on the basis of alterations perceptible with the senses and of the total organism count.

Added preservative	Shelf life [days]
1.1 % of benzoic acid	16
50,000 U of lysozyme/ml + 1.1 % of benzoic acid	33